

Sustained repression and translocation of Ntcp and expression of Mrp4 for cholestasis after rat 90% partial hepatectomy

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Background & Aims: To clarify the mechanism of persistent cholestasis after massive hepatectomy, the relationship between such cholestasis and the expression and localization of organic anion transporters for bile acids was examined in a rat model.

Methods: Male Sprague–Dawley rats were subjected to 90% hepatectomy, and tissues were harvested at 0, 1, 3, and 7 days for microarray analysis, quantitative real-time polymerase chain reaction (RT-PCR), Western blotting, and immunohistochemistry to examine the expression of multidrug resistance protein 4 (Mrp4), bile salt export pump (Bsep), and sodium-dependent taurocholate cotransporting polypeptide (Ntcp).

Results: Persistently elevated levels of serum bile acids were observed at days 3 and 7. RT-PCR and Western blotting indicated that the expression of Mrp4, a bile acid export pump located in the basolateral membrane, was increased at day 3. The expression of Ntcp, a transporter used to uptake bile acids from the sinusoids, was significantly decreased throughout the period. The levels of Bsep, an export pump localized to the canalicular membrane, were unchanged. Immunohistochemistry revealed the localization of Mrp4 and Bsep in the basolateral and canalicular membranes, respectively. On the other hand, at days 3 and 7, Ntcp was localized in the cytoplasm and was hardly detected in the basolateral membrane.

Conclusions: These results suggested that the sustained repression and translocation of Ntcp and the expression of Mrp4 at the basolateral membrane seem to be responsible for the high blood bile acids levels after massive hepatectomy.

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Introduction

In the modern setting of hepatobiliary surgery and adult living donor liver transplantation, a small remnant liver volume is of major concern because this is associated with cholestasis and mortality [1,2]. Cholestasis refers to the suppression of bile secretion, which causes biliary constituents to be retained within hepatocytes and the systemic circulation. In particular, bile acids, the major components of bile, can damage cell membranes because of their detergent properties [3]. It has been suggested that when the intracellular concentration of bile acids exceeds the cytosolic binding capacity, the acids induce apoptosis and necrosis by damaging mitochondria [4]. Therefore, it is crucial to clarify the regulation of the bile acid transport system in small remnant livers.

Hepatocytes efficiently extract bile acids from sinusoidal blood plasma and excrete them into bile canaliculi using organic anion transporters. The hepatic uptake of bile acids from the sinusoids is mainly mediated by the sodium-dependent taurocholate cotransporting polypeptide (Ntcp, gene symbol *Slc10a1*) [5] and the sodium-independent organic anion transporting polypeptide (Oatp1, gene *Slc21a1*) [6], which are located at the basolateral membrane [7,8]. The extracted bile acids are excreted into the bile canaliculi by adenosine triphosphate (ATP)-dependent canalicular transporters, such as the bile salt export pump (Bsep, gene *Abcb11*) [9] and the multidrug resistance protein 2 (Mrp2, gene *Abcc2*) [10]. In addition to these basolateral uptake and canalicular export systems, the basolateral membrane also contains efflux pumps, such as the multidrug resistance protein 3 (Mrp3, gene *Abcc3*), the multidrug resistance protein 4 (Mrp4, gene *Abcc4*), and the organic solute transporter (*Ostα*–*Ostβ*, gene *Ostalpha*–*Ostbeta*), which are normally expressed at very low levels and whose expression levels are upregulated during obstructive cholestasis [11–13].

After partial hepatectomy, the remnant liver is exposed to high bile acid flux, which may aggravate liver injuries. Liver transporters are suggested to play a role in protecting the regenerating liver [14,15] because they are determinants of the intracellular bile acid concentration [16]. However, little is known about how these transporters behave in proliferating hepatocytes and are involved in persistent cholestasis during the recovery phase after massive hepatectomy. We previously reported that

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Abbreviations: Mrp, multidrug resistance protein; Bsep, bile salt export pump; Ntcp, sodium-dependent taurocholate cotransporting polypeptide; Oatp1, sodium-independent organic anion transporting polypeptides; Ost, organic solute transporter; RT-PCR, real-time polymerase chain reaction; Cyp, cytochrome P450; PcnA, proliferating cell nuclear antigen.



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hyperbilirubinemia after 90% hepatectomy was associated with Mrp2 repression and Mrp3 expression [17]. Therefore, our aim in this study was to investigate the regulation and tissue localization of the main transporters for bile acids such as Ntcp, Bsep, and Mrp4, in order to elucidate the association between these transporters and persistent cholestasis. We provide evidence for the sustained repression and translocation of Ntcp and the expression of Mrp4 after massive hepatectomy, which may be linked to the high serum bile acid levels.

Materials and methods

Animals and animal treatment

Male Sprague–Dawley rats aged 6 to 7-weeks purchased from Charles River Japan, Inc. (Tsukuba, Japan) underwent 70% or 90% hepatectomy as described previously [18,19], with 100% survival rates. The rats were sacrificed at days 0, 1, 3, or 7, and blood and tissues were obtained for analysis. We selected the day 0 rats; i.e., those representing the status just before hepatectomy, as controls. Serum total bile acids were measured using an enzymatic assay with Enzabile Auto kits (Sekisui Medical Co., Ltd., Tokyo, Japan). This study was carried out in accordance with the Guidelines for Animal Experimentation of Hiroshima University.

Microarray analysis

Total RNA was extracted from frozen liver samples at days 0, 1, 3, and 7 after 90% hepatectomy with TRIzol reagent (Invitrogen, Carlsbad, CA). Equal amounts of RNA from three individual livers were combined and ten micrograms of RNA were used for biotin-labeled complementary RNA (cRNA). The labeled and fragmented cRNA was subsequently hybridized to the GeneChip Rat Genome 230 2.0 Array (Affymetrix, Santa Clara, CA). Labeling, hybridization, image scanning, and data analysis were performed at Takara Bio Inc. (Shiga, Japan).

Quantitative real-time polymerase chain reaction (RT-PCR)

Complementary DNA (cDNA) was reverse-transcribed from one microgram of total RNA using the Omniscript RT kit (Qiagen, Tokyo, Japan). A MiniOpticon Detection System (Bio-Rad Laboratories, Hercules, CA) and SYBR Green Supermix (Bio-Rad Laboratories) were used for the quantitation of specific messenger RNA (mRNA). The amplification of *ubiquitin C* cDNA was performed to standardize the levels of the target cDNA. Gene-specific primers were designed according to known rat sequences using the Primer3Plus software (Supplementary Table 1). No non-specific PCR products, as detected by melting temperature curves, were found in any case. After normalizing the expression of the target gene to *ubiquitin C* expression, the level of the expressed mRNA in each sample was expressed relative to the control values.

Western blot analysis

Crude liver membranes were prepared according to the method of Gant *et al.* [20] and preparations (100 μ g protein each) were dissolved in sample buffer and loaded onto a 7.5% or 10% sodium dodecyl sulfate polyacrylamide electrophoresis

gel with a 4.4% stacking gel. After electrophoresis, the proteins were transferred to polyvinylidene fluoride membranes (Hybond-P; GE Healthcare, Buckinghamshire, UK). After being blocked, the membranes were incubated overnight at 4 °C with the following concentrations of the primary antibodies: anti-Mrp4 antibody (1:500, a gift from Dr. John D Schuetz), anti-Bsep antibody (1:500, Abcam, Cambridge, MA), anti-Ntcp antibody (1:10,000, Santa Cruz Biotechnology, Santa Cruz, CA), and anti-Ost α antibody (1:10,000, Santa Cruz Biotechnology). Immune complexes were detected using a species-appropriate horseradish peroxidase-conjugated secondary antibody and an enhanced chemiluminescent kit (ECL Plus; GE Healthcare). Immunoreactive bands were quantified by densitometry (ImageJ software). After normalizing the individual protein bands to a membrane protein band as an internal reference, the protein levels were expressed relative to the control values.

Immunofluorescence microscopy

Before excising the liver specimens, the rats were perfused with physiologic saline followed by 4% paraformaldehyde via the left ventricle. The perfused liver specimens were fixed in 4% paraformaldehyde for two nights and then embedded in paraffin. These paraffin blocks were sliced into 4 μ m thick sections for hematoxylin-eosin staining and Mrp4, Ntcp, and Pcn staining. For immunohistochemical staining, the tissue sections were treated with citrate buffer (Target Retrieval Solution; Dako, Kyoto, Japan) at 121 °C for 3 min. For Bsep staining, small cubes of fresh liver tissue were embedded in OCT compound (Sakura, Tokyo, Japan), and 7 μ m frozen sections were fixed with 4% paraformaldehyde for 5 min. The slides were blocked and then incubated overnight at 4 °C with the primary antibodies against Mrp4, Ntcp, and Pcn (diluted 1:200), and against Bsep (diluted 1:50). After the washings, the slides were incubated with species-appropriate Alexa 488 IgG or/and Alexa 546 IgG (Invitrogen). Images were captured with an

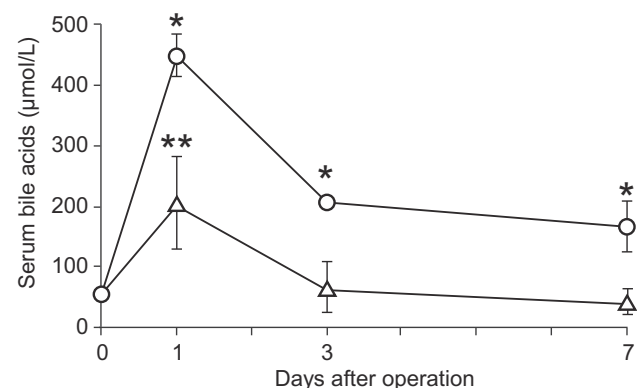


Fig. 2. Levels of serum total bile acids at days 0, 1, 3, and 7 after 70% (triangles) or 90% (circles) hepatectomy. The serum total bile acid level was quantified with a commercial kit. Data are the mean $\pm$ SD from three rats. * p < 0.05 vs. the value in the 70% hepatectomy group; ** p < 0.05 vs. the day 0 value.

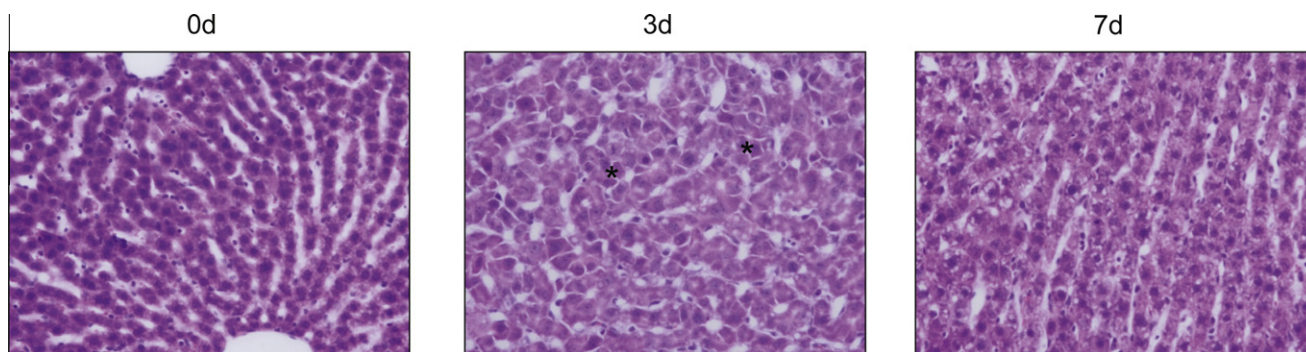


Fig. 1. Hematoxylin-eosin staining of liver sections at days 0 (0d), 3 (3d), and 7 (7d) after 90% hepatectomy. Paraformaldehyde-fixed liver sections were stained with hematoxylin-eosin as described in Section 2. The asterisks on the day 3 panel show clusters of hepatocytes devoid of sinusoidal architecture.

Table 1. The results of microarray analysis.

Cellular function and gene name	gene symbol	Signal			
		Day 0	Day 1	Day 3	Day 7
DNA replication					
proliferating cell nuclear antigen	Pcna	390	1870	790	640
DNA ligase I	Lig1	41	180	77	57
Cell proliferation					
cyclin A2	Ccna2	34	260	250	34
cyclin G1	Ccng1	115	420	210	140
Fatty acid synthesis					
ATP citrate lyase	Acly	220	320	1000	820
acetyl-coenzyme A carboxylase	Acac	170	180	510	450
Cholesterol synthesis					
3-hydroxy-3-methylglutaryl-Coenzyme A reductase	Hmgcr	200	600	1400	890
cytochrome P450, subfamily 51	Cyp51	470	460	2100	1300
Liver-specific protein					
transthyretin (prealbumin)	Ttr	6600	4900	5500	6700
coagulation factor 2	F2	5300	2900	4500	6600
Transporters					
solute carrier family 10 (sodium/bile acid cotransporter family), member 1	Slc10a1	3700	350	1800	3200
solute carrier family 21, member 1	Slc21a1	710	160	480	590
ATP-binding cassette, sub-family B (MDR/TAP), member 11	Abcb11	1700	950	1400	1400
ATP-binding cassette, sub-family C (CFTR/MRP), member 2	Abcc2	620	270	600	850
ATP-binding cassette, sub-family C (CFTR/MRP), member 3	Abcc3	54	48	91	53
Bile acid synthesis					
cytochrome P450, family 8, subfamily b, polypeptide 1	Cyp8b1	3400	2700	1500	150
cytochrome P450, family 27, subfamily a, polypeptide 1	Cyp27a1	2600	440	1400	1900
Conjugation					
liver UDP-glucuronosyltransferase	Udpgtr2	2600	820	2000	2500
bile acid-Coenzyme A: amino acid N-acyltransferase	Baat	4000	1200	2700	4200
Proteasome					
proteasome (prosome, macropain) subunit, beta type 8	Psmb8	160	300	630	500
proteasome (prosome, macropain) subunit, beta type 9	Psmb9	110	240	820	390

Heavily shaded area means more than twofold higher signal than on day 0. Lightly shaded area means less than half the signal that on day 0.

Olympus IX71 fluorescent microscope (Olympus Optical, Tokyo, Japan) or a Zeiss LSM710 confocal scanning microscope (Zeiss, Wetzlar, Germany). Digital images were processed with Adobe Photoshop (Adobe, San Jose, CA).

Statistical analysis

Data are presented as means $\pm$ SD. Differences between experimental groups were assessed for significance using the two-tailed unpaired Student's *t*-test. *p* values <0.05 were considered to be statistically significant.

Results

Restoration of liver weight and lobule structure

At days 1 and 3, the liver weight of the 90% hepatectomy rats was significantly lower than that of the 70% hepatectomy rats (Supplementary Fig. 1). At day 3, the weight of the remnant liver after 90% hepatectomy was only 26% of that at day 0. At day 7, liver weight had reached 55% of the value at day 0 in the 90% hepatectomy group and 58% in the 70% hepatectomy group, and the difference between 70% and 90% hepatectomy was not significant. The alterations in hepatic lobule structure during the regenera-

tive process in 90% hepatectomy are shown in Fig. 1. At day 3, clusters of hepatocytes were seen sporadically (asterisks in Fig. 1), suggesting that hepatocyte proliferation continued and that the sinusoidal architecture had not completely formed. At day 7, the structure of the sinusoids had almost returned to normal; i.e., they passed between the plates of hepatocytes, with individual cells being exposed to blood on two sides.

Persistently elevated serum bile acids

Serum bile acid levels initially increased at day 1 in both hepatectomy groups, but the level was higher in 90% hepatectomy (Fig. 2). At day 3, it had returned to the level seen at day 0 in 70% hepatectomy, whereas it remained high in 90% hepatectomy and the higher value was maintained at day 7.

Gene expression profiles during regeneration after 90% hepatectomy

At first, in order to understand the relationship between cell proliferation and liver specific functions, including bile acid metabolism during regeneration after 90% hepatectomy, gene expression

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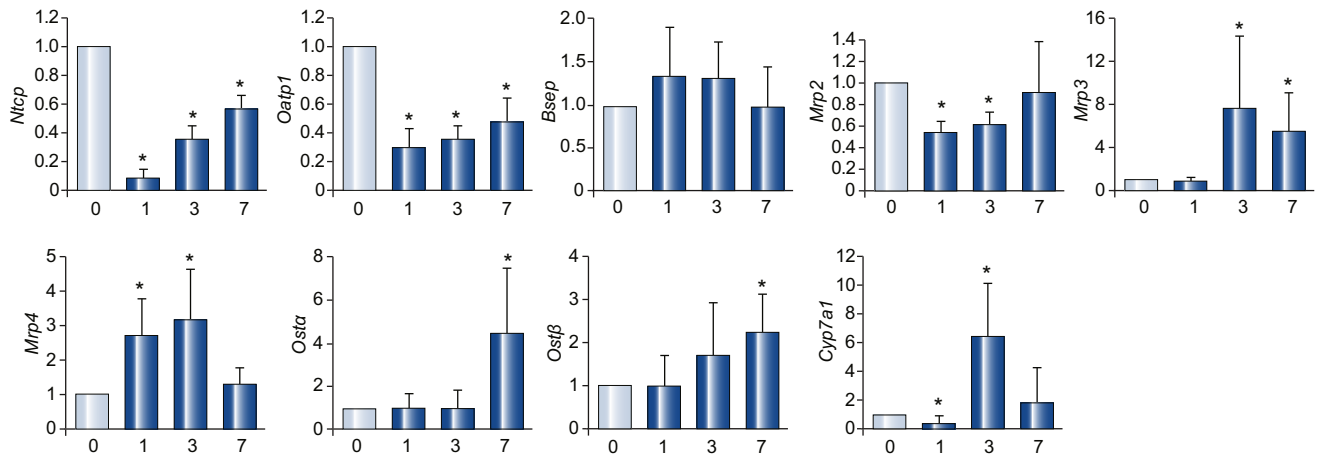


Fig. 3. The quantitation of mRNA for organic anion transporters and Cyp7a1 at days 0, 1, 3, and 7 after 90% hepatectomy. The quantitation of *Bsep*, *Mrp2*, *Mrp3*, *Mrp4*, *Ntcp*, *Oatp1*, *Ostα*, *Ostβ*, and *Cyp7a1* mRNA was performed by RT-PCR, employing specific primers, as described in Section 2. The levels of mRNA at days 1, 3, and 7 (closed bars) are expressed relative to the values of individual mRNA at day 0 (open bars). Data are the mean $\pm$ SD from three rats. * $p < 0.05$ vs. the day 0 value.

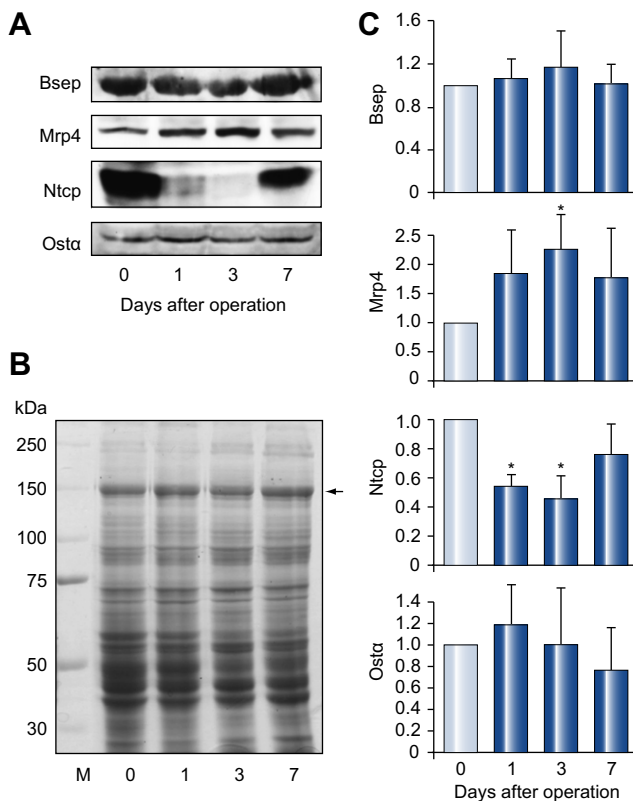


Fig. 4. Western blot analysis of liver tissue membrane fractions at days 0, 1, 3, and 7 after 90% hepatectomy. The procedures used for the preparation of the liver membrane fractions and Western blotting are described in Section 2. (A) Western blotting results for *Bsep*, *Mrp4*, *Ntcp* and *Ostα*. Each lane contains 100 μ g of protein. Data are representative of 3 independent experiments. (B) Protein staining with Coomassie Brilliant Blue. An arrow indicates a protein band used as an internal reference for protein normalization. Lane M, molecular mass marker proteins. The numbers on the left indicate molecular mass in kDa. (C) Quantitation of *Bsep*, *Mrp4*, *Ntcp*, and *Ostα* during the regeneration period. The Western blotting bands were quantified with a scanning densitometer and normalized as described in Section 2. The levels of proteins at days 1, 3, and 7 (closed bars) are expressed relative to the values of individual proteins at day 0 (open bars). Data are the mean $\pm$ SD from three rats. * $p < 0.05$ vs. the day 0 value.

profiles were examined by microarray analysis. Data are presented as signal values and values above the twofold threshold, as compared with the value at day 0, were defined as indicating significant and reproducible alterations in gene regulation [21] (Table 1).

Following hepatectomy, the remaining liver showed upregulated expression of genes involved in DNA replication and cell proliferation in accordance with the results for liver weight at days 1 and 3. Genes for fatty acid and cholesterol synthesis were also upregulated at days 3 and 7. *Ntcp* (*Slc10a1*) was downregulated at days 1 and 3, and *Oatp1* (*Slc21a1*) and *Mrp2* (*Abcc2*) were also repressed at day 1. *Bsep* (*Abcb11*) was unchanged during the regeneration period. Contrary to the upregulation of the cholesterol synthesis genes, cytochrome P450 8b1 (*Cyp8b1*) was downregulated at days 3 and 7, consistent with the findings of a previous study [22]. Proteasome subunit genes *Psmb8* and *Psmb9* were upregulated at days 3 and 7.

Changes in organic anion transporter mRNA levels after 90% hepatectomy

Next, to confirm the microarray results, the mRNA levels of the organic anion transporters for bile acids in the 90% hepatectomy samples were quantified by RT-PCR (Fig. 3). *Ntcp* and *Oatp1* mRNA were significantly decreased to 10.0 ± 5.7 and 31.2 ± 12.4 , 35.4 ± 6.5 and 36.5 ± 9.4 , and 56.9 ± 9.5 and $49.5 \pm 15.2\%$ of the controls at days 1, 3, and 7, respectively. *Mrp2* mRNA was also decreased to 55.1 ± 8.7 and $60.6 \pm 11.1\%$ of the control at days 1 and 3, respectively. *Mrp4* mRNA was increased to 272 ± 105 and $317 \pm 150\%$ of the control at days 1 and 3, and *Mrp3* mRNA increased to 774 ± 677 and $548 \pm 350\%$ at days 3 and 7, respectively, *Ostα* mRNA increased to 441 ± 302 at day 7 and *Ostβ* mRNA increased to 231 ± 90 at day 7. No alterations in the *Bsep* mRNA level were observed. The mRNA level of cytochrome P450 7a1 (*Cyp7a1*), the rate-limiting enzyme of the bile acid synthesis pathway, was decreased to $45.8\% \pm 35.9\%$ at day 1 but increased to $630\% \pm 347\%$ at day 3.

Increased Mrp4 protein and decreased Ntcp after 90% hepatectomy in Western blotting

To examine whether the changes in *Bsep*, *Mrp4*, *Ntcp* and *Ost α* mRNA expression were associated with changes in the levels of the respective proteins, Western blotting was performed using membrane fractions from the 90% hepatectomy samples. As shown in Fig. 4, *Ntcp* protein levels were significantly decreased to 54.5 ± 8.2 and $45.3 \pm 17.0\%$ of the control on days 1 and 3, respectively. The *Mrp4* level was increased to $221 \pm 59\%$ of the control on day 3. *Bsep* and *Ost α* protein did not change throughout the regeneration period. These protein changes were almost in line with the changes in mRNA expression.

Translocation of Ntcp into the cytoplasm after 90% hepatectomy

The tissue distributions of *Bsep*, *Mrp4*, and *Ntcp* in 90% hepatectomy samples were examined by immunofluorescence microscopy. As shown in Fig. 5, *Bsep* was localized at the canalicular membranes of hepatocytes throughout the period. Nuclear staining seemed to be nonspecific, because a similar staining pattern was seen with nonimmune γ -globulin (*Bsep* inset, Fig. 5). *Mrp4*

staining was increased during the period but was restricted to the basolateral membrane. On the other hand, although *Ntcp* was localized at the basolateral membrane at day 0, it was diffusely distributed in the cytoplasm of many hepatocytes at day 3. At day 7, *Ntcp* was stained as large granules scattered throughout the cytoplasm (Fig. 5, arrowheads in the *Ntcp* panel at day 7) and in part of the basolateral membrane (arrows in the same panel). To further examine the alterations in *Ntcp* localization during the regeneration process, its expression was compared with the expression of *Mrp4* by two-color analysis. This analysis clearly showed the different localizations of *Ntcp* and *Mrp4* at day 3: *Ntcp* being present in the cytoplasm and *Mrp4* in the basolateral membrane with no overlapping between them (Fig. 6A). At day 7, most *Ntcp* and *Mrp4* still displayed different localizations, but the merged image revealed some overlapping in part of the basolateral membrane (Fig. 6A, yellow). Thus, *Ntcp* presented the ability to translocate into the cytoplasm and a delay in the recovery of its expression in the basolateral membrane. To examine whether changes in *Ntcp* localization occurred in proliferating hepatocytes, *Ntcp* expression was examined in cells expressing proliferating cell nuclear antigen (Pcna) (Fig. 6B). At day 3, many cells were positive for Pcna, and *Ntcp* was distributed

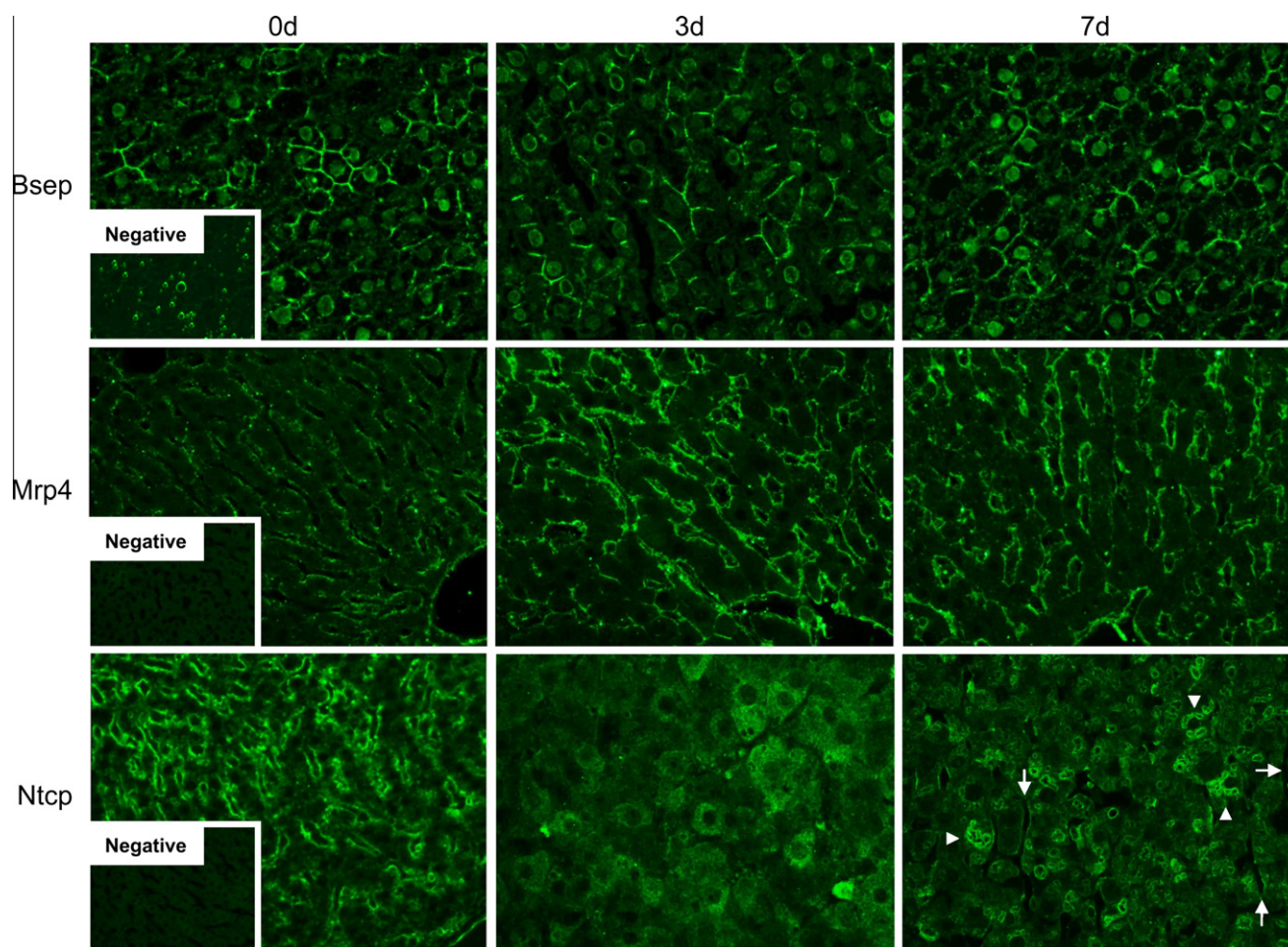


Fig. 5. Immunofluorescent localization of *Bsep*, *Mrp4*, and *Ntcp* at days 0 (0d), 3 (3d), and 7 (7d) after 90% hepatectomy. Immunofluorescence microscopy was performed as described in Section 2. The respective primary antibody and Alexa 488-labeled secondary antibody (green) were used. No immune γ -globulin was used instead of the primary antibodies for the negative controls (insets in 0d). The arrows and arrowheads indicate the staining of the basolateral membrane and cytoplasm, respectively. Original magnification: 200 $\times$.

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as fine spots in the cytoplasm of the PcnA-positive cells. PcnA staining was negative at day 7, and Ntcp was concentrated as large granules in the cytoplasm near to the plasma membrane.

Discussion

To clarify the mechanism of persistent cholestasis after massive hepatectomy, alterations in factors involved in bile acid metabolism during the regeneration period were examined using microarray, RT-PCR, Western blotting, and immunofluorescence microscopy. Expression array analysis revealed maximal DNA replication at day 1 and enhanced cell proliferation at days 1 and 3, and these results were in line with the findings of hematoxylin-eosin staining.

The present study showed significant decreases in the *Ntcp* mRNA level at days 1, 3, and 7 after 90% hepatectomy, in accordance with previous studies reporting the downregulation of *Ntcp* mRNA and protein levels during the early phase of rat liver regeneration after 70% partial hepatectomy [14]. However, in contrast to what observed in 70% hepatectomy, the levels of serum bile acids at day 7 after 90% hepatectomy remained higher than those at day 0, despite the restoration of liver weight and lobular structure. Besides the downregulation of Ntcp protein in 90% hepatectomy, immunofluorescence microscopy revealed that most Ntcp was localized in the cytoplasm, even at day 7, and only a small fraction was present at the basolateral membrane. These results suggest that the Ntcp present in the cytoplasm is not functional as a transporter for bile acids and that poor uptake of these compounds from the basolateral membrane may be responsible for the higher serum bile acid levels. In spite of the marked down-

regulation of *Ntcp* mRNA at days 1 and 3, the protein was present as fine spots in the cytoplasm. As the amount of Ntcp protein was very high at day 0, as shown by Western blotting and immunofluorescence microscopy, the cytoplasmic localization of the protein suggested a possible degradation process rather than synthesis. In this context, in microarray analysis, some genes encoding proteasome subunits were upregulated at day 3. It was also reported that wild-type rat Ntcp is degraded by the ubiquitin-proteasome system [23]. Moreover, similar endocytotic translocation of Ntcp was reported after treatment with a mitogen, phorbol myristate acetate [24]. At day 7, Ntcp was found as large granules in the cytoplasm near the plasma membrane. The relative recovery of the *Ntcp* mRNA level and positive Western blotting data raised the possibility that the Ntcp-positive granules might represent exocytotic vesicles containing newly synthesized Ntcp to be transported to the basolateral membrane. These results indicated a delay in the recovery of Ntcp-mediated bile acid uptake after massive hepatectomy. The downregulation of Ntcp expression may be favorable for hepatocyte regeneration and the subsequent reorganization of lobule structure as it helps to avoid the toxic effects of bile acids [4,24].

Mrp4 mRNA and protein were upregulated after 90% hepatectomy, and the protein was localized at the basolateral membrane throughout the regeneration period. This finding was in clear contrast to the cytoplasmic localization of Ntcp. We previously reported an increase in Mrp3 and a decrease in Mrp2 protein levels at day 3 after 90% hepatectomy [17], and the present study confirmed these findings. Although Mrp3 is present in the basolateral membrane, a study employing *Mrp3*-null mice showed that it was not a significant determinant of the serum bile acid level [25]. On the other hand, as Mrp4 is an ATP-dependent pump

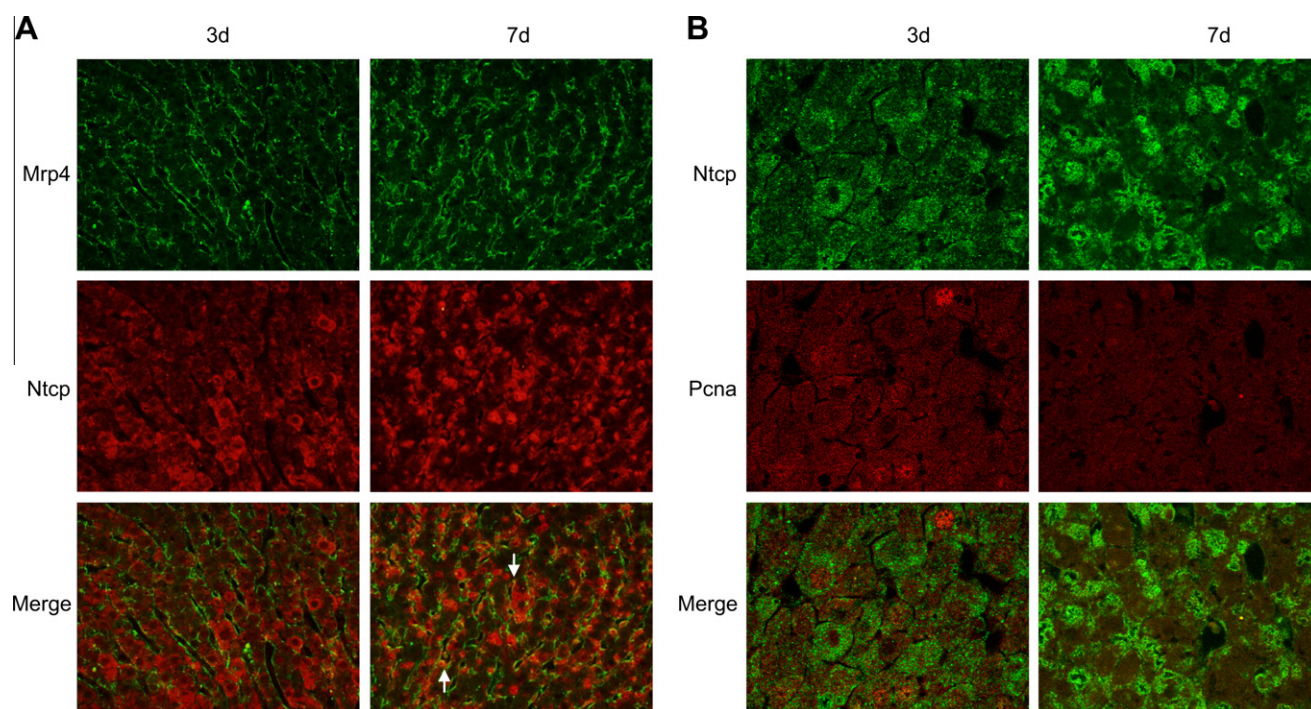


Fig. 6. Immunofluorescence staining of Ntcp and Mrp4, and Ntcp and PcnA on days 3 (3d) and 7 (7d) after 90% hepatectomy. (A) Cytoplasmic localization of Ntcp at days 3 and 7. The Mrp4-Alexa 488 (green), Ntcp-Alexa 546 (red), and merged signals are shown. At day 7, some overlapping between Ntcp and Mrp4 was observed in the basolateral membrane (arrows). Original magnification: 200 $\times$. (B) Cytoplasmic localization of Ntcp in proliferating hepatocytes. The Ntcp-Alexa 488 (green), PcnA-Alexa 546 (red), and the merged image are shown. Original magnification: 400 $\times$.

that exports bile acids from hepatocytes into the blood [26], the upregulation and localization of Mrp4 at the basolateral membrane, in addition to the downregulation of Ntcp function, seem to be responsible for the high blood bile acid levels during the regeneration period. Although Ntcp, the main transporter for bile acid uptake, was downregulated, Mrp4 induction raised the possibility of enhanced intracellular bile acid concentrations. As the expression and localization of Bsep were not changed, the canalicular export system seemed to be maintained during the regeneration period. The induction of Cyp7a1 suggested increased synthesis of bile acids in hepatocytes. Thus, this may be involved in a possible high intracellular bile acid level. However, the disproportion between the export and uptake of bile acids may also determine the levels inside hepatocytes. Further studies are needed to clarify the contribution of synthesis and transport to the intracellular bile acid level during the regeneration process.

Considering these alterations in the transport of bile acids in hepatocytes during the regeneration period, in order to compensate for the downregulation of Ntcp activity, the activation of alternative excretory routes may prevent the systemic dysfunction caused by high blood bile acid levels [27]. The kidney Mrp2 protein is a possible candidate for such an alternative pathway because it has been reported to function as an efflux pump into urine [28].

In summary, the persistent elevation of serum bile acids after massive hepatectomy was suggested to be due to sustained low uptake from the sinusoids by repressed and translocated Ntcp and increased export from hepatocytes into the blood by Mrp4 in the basolateral membrane.

Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhep.2010.11.023.

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